CLAIMS

1. A method of transfer of a gene of interest to a product vector comprising:

a) introducing into a prokaryotic host cell which expresses a gene encoding a site-specific recombinase:

a first vector comprising:

a gene of interest,

a gene encoding a first selectable marker,

a double-stranded origin of replication of a rolling circle replicon; and

a site-specific recombination recognition site, wherein said gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

a second vector comprising:

a negative selectable marker,

a double-stranded origin of replication of a rolling circle replicon,

a site-specific recombination recognition site,

a single-stranded origin of replication, and

a gene encoding a second selectable marker, wherein said negative selectable marker is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site;

wherein said host cell further expresses a gene encoding a rep protein that can initiate replication as said double stranded origins of replication, and wherein said introducing permits formation of a product vector comprising said gene of interest interposed between said double-stranded origin of replication of said second vector and said site-specific recombination recognition site, said single-stranded origin of replication of said second vector, and said gene encoding said second selectable marker, said product vector not including both of said negative selectable marker and said gene encoding said first selectable marker.

2. The method of claim 1, wherein said prokaryotic host cell is grown under conditions which permit said first and second vectors to recombine to form a co-integrate vector.

3. The method of claim 1, wherein said product vector is isolated from said prokaryotic host cell.

4. A pair of vectors comprising:

(a) a first vector comprising:

a gene of interest,

a gene encoding a first selectable marker,

a double-stranded origin of replication of a rolling circle replicon; and

a site-specific recombination recognition site, wherein said gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

(b) a second vector comprising:

a negative selectable marker,

a double-stranded origin of replication of a rolling circle replicon,

a site-specific recombination recognition site,

a single-stranded origin of replication, and

a gene encoding a second selectable marker, wherein said gene encoding said negative selectable marker is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site,

wherein in one or both of said first and second vectors there is no second site-specific recombinase recognition site between said double-stranded origin of replication and said site-specific recombinase recognition site.

5. The vectors of claim 4, wherein said first selectable marker and said second selectable marker are different.

6. The vectors of claim 4, wherein said site-specific recombinase recognition site is selected from the group consisting of: *loxP, loxP2, loxP3, loxP23, loxP511, loxB, loxC2, loxL, loxR, loxΔ86, loxΔ117, frt, dif*, λ-phage *att* sites, and ΦC31 *att* sites.

7. The vectors of claim 4, wherein said double-stranded origin of replication is the double-stranded origin of replication of the filamentous bacteriophage f1.

8. The vectors of claim 4, wherein said double-stranded origin of replication is the double-stranded origin of replication of the plasmid pKym.

9. The vectors of claim 4, wherein said negative selectable marker is selected from the group consisting of: *rpsL* and *sacB*.

10. The vectors of claim 4, wherein said gene encoding one of said first or second selectable markers, independently, is selected from the group consisting of: kanamycin resistance gene, the ampicillin resistance gene, the spectinomycin resistance gene, the gentamycin resistance gene, the tetracycline resistance gene, the chloramphenicol resistance gene, the streptomycin resistance gene, the *strA* gene, and the *sacB* gene.

11. A product vector comprising:

a gene of interest;

a double-stranded origin of replication of a rolling circle replicon

a site-specific recombination recognition site

a single-stranded origin of replication; and

a nucleic acid sequence encoding a second selectable marker;

wherein said gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site.

12. A kit for the transfer of a gene of interest to a product vector comprising:

(a) a first vector comprising:

a gene of interest,

a gene encoding a first selectable marker,

a double-stranded origin of replication of a rolling circle replicon; and

a site-specific recombination recognition site, wherein said gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

(b) a second vector comprising:

a negative selectable marker,

a double-stranded origin of replication of a rolling circle replicon,

a site-specific recombination recognition site,

a single-stranded origin of replication, and

a gene encoding a second selectable marker, wherein said negative selectable marker is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

packaging materials therefore,

wherein in one or both of said first and second vectors there is no second site-specific recombinase recognition site between said double-stranded origin of replication and said site-specific recombinase recognition site.

13. A kit for the transfer of a gene of interest to a product vector comprising:

(a) a first vector comprising:

a cloning site for insertion of a gene of interest,

a gene encoding a first selectable marker,

a double-stranded origin of replication of a rolling circle replicon; and

a site-specific recombination recognition site, wherein said cloning site for insertion of a gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

(b) a second vector comprising:

a negative selectable marker,

a double-stranded origin of replication of a rolling circle replicon,

a site-specific recombination recognition site,

a single-stranded origin of replication, and

a gene encoding a second selectable marker, wherein said negative selectable marker is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

packaging materials therefore,

wherein in one or both of said first and second vectors there is no second site-specific recombinase recognition site between said double-stranded origin of replication and said site-specific recombinase recognition site.

14. The kit of claim 12 or 13, wherein said kit further comprises a primary host cell which supports replication of a vector having a rolling circle double-stranded origin of replication and which possesses a site-specific recombinase specific for said site-specific recombination site..

15. The kit of claim 12 or 13, wherein said kit further comprises a site-specific recombinase.

16. The kit of claim 14, said host cell being transfectable.

17. The kit of claim 12 or 13, further comprising a secondary host cell.

18. The kit of claim 12 or 13, further comprising *in vitro* recombination buffer.

19. A pair of vectors comprising:

(a) a first vector comprising:

a cloning site for insertion of a gene of interest,

a gene encoding a first selectable marker,

a double-stranded origin of replication of a rolling circle replicon; and

a site-specific recombination recognition site, wherein said cloning site for insertion of a gene of interest is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site; and

(b) a second vector comprising:

a negative selectable marker,

a double-stranded origin of replication of a rolling circle replicon,

a site-specific recombination recognition site,

a single-stranded origin of replication, and

a gene encoding a second selectable marker, wherein said negative selectable marker is interposed between said double-stranded origin of replication of a rolling circle replicon and said site-specific recombination recognition site,

wherein in one or both of said first and second vectors there is no second site-specific recombinase recognition site between said double-stranded origin of replication and said site-specific recombinase recognition site.